

2012

# Effects of leaf litter diversity on nutrients and mosquito communities in Neotropical artificial tree holes

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# EFFECTS OF LEAF LITTER DIVERSITY ON NUTRIENTSAND MOSQUITO COMMUNITIES IN NEOTROPICAL ARTIFICIAL TREE HOLES

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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July 31, 2012

## Acknowledgment

Thank you to my advisor Dr. James Vonesh for providing excellent advice on academics, life, and science as well as believing in me when I was not able to believe in myself. I am extremely lucky to have had an advisor as hands-on, enthusiastic, and understanding as you are and I will always be grateful.

I was also lucky to have a second mentor when I came to VCU. Dr. Johanna Kraus received the brunt of my questions when I first came to VCU and I thank her for her patience and understanding when I was constantly pestering. She also opened my eyes to the complexities of the terrestrial and aquatic interface.

I would also like to thank Julie Charbonnier, Lindsey Wargelin, Kristina Cohen, and Maria José Salica for tireless enthusiasm, amazing mojitos, and the best leaf cutting parties in the northern hemisphere. Julie Charbonnier gets special thanks for her support and understanding at all hours of the night and day. Thanks also go out to the Graduate Organization of Biology Students for their friendship and camaraderie throughout graduate school.

Last but not least, I would like to thank Shawn Hill and my family for always being there for me whenever I needed them. I could not have finished without your support.

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# Abstract

**EFFECTS OF LEAF LITTER DIVERSITY ON NUTRIENTS AND MOSQUITO COMMUNITIES IN NEOTROPICAL ARTIFICIAL TREE HOLES**

By Rachel Komosinski, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

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Director: James R. Vonesh, Ph.D. Assistant Professor, Department of Biology

Inputs from terrestrial habitats to aquatic habitats are important for structuring aquatic communities. Terrestrial producer diversity in the tropics may decline due to anthropogenic causes. I investigated how tree diversity affects aquatic communities. We used leaves from three timber-producing species (*Dalbergia retusa*, *Pachira quinata*, and *Tectona grandis*) to test the effects of leaf litter species composition and richness on invertebrate aquatic communities in Gamboa, Panama. We quantified macroinvertbrate species richness and abundances, leaf litter mass loss, and dissolved carbon to nitrogen ratios (C:N) after 4 weeks. We found that litter types differed in breakdown and C:N. *Tectona grandis* had lower dissolved C:N than both native species and supported the fewest number of invertebrates. C:N ratios declined with increasing litter diversity; however breakdown was not affected by litter richness. Mosquito abundance

increased with litter species richness. Results of this study highlight the importance of diverse detritus in structuring aquatic treehole communities.

# INTRODUCTION

Terrestrial systems have significant effects on the aquatic communities embedded within them through characteristics of the terrestrial matrix and the resources they contribute to the aquatic community as allochthonous inputs (Behmer & Hawkins 1986, Joly et al. 2001, Rubbo et al. 2006). Canopy cover and temperature, products of habitat structure, are large drivers of habitat selection and performance for the vertebrate and invertebrate species in aquatic habitats embedded within forested areas (e.g. beetles: Binckley & Resetarits 2007; fish: Power 1984; amphibians: Werner & Glennemeier 1999). Many aquatic communities also depend on plant and animal material from the terrestrial community (e.g. lakes: Pace et al 2004; pitcher plants: Miller & Kneitel 2005). The quality and quantity of these inputs can be important to the system for colonization and consumption by detritivores (e.g. streams: Motomori et al. 2001; ponds: Williams et al. 2008).

Leaf litter manipulation studies are common and communities of interest can vary in complexity from microbes to zooplankton to vertebrates. Effects of leaf litter are not consistent across taxa as some groups are more highly affected than others (Warren & Spencer 1998; Rubbo et al. 2008). Responses to leaf litter are also specific to the type/identity of leaf litter used; for instance larval wood frogs (*Rana sylvatica*) respond negatively to red maple leaves when

compared with oak leaves, making forest composition an important factor in the effects of terrestrial inputs on aquatic systems (Rubbo & Kiesecker 2004).

Terrestrial producer diversity can decline as a result of both anthropogenic modifications of the environment (e.g. land use; Leigh et al. 1993, Vitousek et al. 1997) and natural processes, such as monotypic forest stands produced by intense competition or clearing by other organisms (Torti et al. 2001, Frederickson et al. 2005). While rates of deforestation are expected to decrease, tropical forests are projected to continue to lose forested area (Wright & Muller-Landau 2006). Loss of species in old growth forests due to deforestation can be partly ameliorated by planting of successional forests; but, this may result in a decrease in species richness with continued deforestation (Dent & Wright 2009). There has been recent positive movement towards sustainable forestry management and ecosystem-based management practices in which foresters investigate not only ways to increase timber productivity but also ways that maintain or improve ecosystem services (Schlaepfer & Ellito 2000). Interactions between species in mixed-species plantations results in different resource use and changes in productivity compared to monocultures (Richards et al. 2010). Loss of terrestrial diversity can affect terrestrial arthropod diversity and ecosystem services in tropical systems (Lawton et al 1998, Ruiz-Jaen & Potvin 2010) and has effects moving up trophic levels (Siemann 1998, Haddad et al. 2009). These effects of producer diversity may transfer to detritally-driven aquatic communities.

Understanding how producer diversity affects communities has become an increasingly important theme in ecology both in “green” (primary producer) and “brown” (detrital) pathways. Decomposition patterns driven by detritus from producers are different in comparison to patterns in production (Cardinale et al. 2011). Across most systems there is a significant effect of

diversity on production; however, in decomposition, of the three general systems studied (grassland, temperate forest, and stream), only studies in streams have shown a consistent effect of detrital diversity on decomposition rates (Cardinale et al. 2011). Leaf litter breakdown is shown to behave in a non-additive fashion in streams, with polycultures losing litter faster than expected and higher levels of richness causing increased decomposition rates (Swan & Palmer 2004, Leroy & Marks 2006). These effects of detrital diversity can also move up into consumers; in streams, higher leaf litter diversity can result in different invertebrate assemblages than expected when compared to single species assemblages (Leroy & Marks 2006) and effects on shredders may mediate the effects of diversity on decomposition (Sanpera-Calbet et al. 2009). However, the effects on invertebrate communities are incongruent and sometimes result in opposite trends in communities. For example, Taylor et al. (2007) found only minimal enhancement of decomposition by litter mixing and virtually no effect of mixing on benthic invertebrate abundance or richness.

Water-filled tree holes, one type of phytotelma ('plant-held water'), are detritally-based systems that are highly affected by leaf litter input (Kitching 2001). Variation in leaf litter quality and quantity are important for survival, development, and size of inhabitants (Walker et al. 1997). Detritus and water stemflow from trees results in nutrient-rich water and bacterial populations that support higher trophic levels (Walker et al. 1991). In addition to detrital input, communities in isolated water containment habitats are affected by land use changes in the surrounding terrestrial matrix. For example, habitat specialization of insects results in more similar forest communities across detritus levels than those with heavier human presence like prairie or urbanized habitat (Yanoviak 2006, Yee & Yee 2007). Biotic interactions within communities are also important; due to their small size and isolation, predators in treeholes can

reduce invertebrate abundance and lead to localized extinction in some cases, (Fincke et al. 1997; Yanoviak 2001b). Tree hole communities also lend themselves to questions in metacommunity dynamics due to their temporal and spatial variabibility (Ellis et al 2006). They harbor predominantly invertebrate communities composed of obligate and preferential tree-hole residents with complex life cycles, mainly dipterans and beetles (Verdonschot et al. 2008).

Mosquito larvae are the principal dipteran inhabitants in treeholes and container habitats; globally, average mosquito richness in a given treehole varies from 0.30 species in Britain to 2.4 species in Kenya (2005). Some mosquito species are efficient vectors for human disease pathogens. Therefore dynamics in aquatic habitats that house larval mosquitoes have implications for human health. Changes in these aquatic communities due to anthropogenic modifications of tree diversity could have important cascading impacts on pathogen vectors (Norris 2004). Habitat disturbances in New Zealand favor invasive vector mosquitoes and negatively impact native mosquitoes (Derraik & Slaney 2007). Reiter and LaPointe (2007) found that in Hawai'i the vector mosquito *Culex quinquefasciatus* was more abundant in forest fragments and farmland than in intact forests. Tropical countries are especially vulnerable for vector borne diseases due to high temperatures and rainfall; however, the Intergovernmental Panel on Climate Change predicts warming trends that would expand current ranges for vectors and their pathogens (McMichael & Haines 1997).

Understanding the significance of terrestrial and aquatic linkages, considering reductions in tropical tree diversity, and knowing that plant species loss can affect invertebrate communities, it is important that we investigate how tree diversity affects the aquatic communities embedded within tropical forests. Studies that have explored the effects of tree diversity on aquatic communities have mainly concentrated on changes in temperate stream

detritivore communities due to effects of leaf litter quantity or quality (Leroy & Marks 2006; Kominoski et al 2009). Small, temporary waters, such as water-filled tree holes, are understudied in this regard. Building upon previous studies in this region (Fincke et al 1997; Yanoviak 1999; Yanoviak 2001a, b, c; Yanoviak et al 2006), we investigated the effects of leaf litter type and diversity on litter mass loss, dissolved organic nutrients, total invertebrate abundance, and abundances of common mosquito species in tropical water-filled tree holes. Specifically, we addressed two main questions in regard to these responses: 1) Do responses differ across litter type/identity? 2) And is there an effect of leaf litter diversity upon these responses? We expected tree species known to have high quality leaf litter (low C:N ratios based on previous studies) would result in water with low dissolved C:N ratios as well as more leaf litter loss and higher invertebrate abundances.

## MATERIALS AND METHODS

*Study System*

The Agua Salud Project, a joint effort among the Panama Canal Authority (ACP), the National Environmental Authority of Panama (ANAM), and the Smithsonian Tropical Research Institute (STRI), aims to determine how land use alters ecosystem services. The project is seeking to understand the effects of land use on the Panama Canal Watershed and to optimize the intersection between forest/timber production and environmental services provided by forested lands (Van Breugel & Hall 2008). One goal is to investigate the effects of native timber species plantations in mixtures and monocultures as well as comparing them to plantations with the common, exotic species, *Tectona grandis* (Teak; abbreviated Te). We placed our question on the effects of leaf litter diversity on tree holes within a forest management perspective. Therefore, we used leaves from two of the native species from the Agua Salud study, *Dalbergia retusa* (Cocobolo; abbreviated Co) and *Pachira quinata* (Spiny Cedar; abbreviated Sp) as well as the exotic Teak. Teak has increased globally in planted area and most recent estimates show that it composes 49% of agroforestry in Panama (FAO Teak Assessment). Cocobolo is a nitrogen-fixing leguminous tree typically used for carving/artisanal woodwork while Spiny Cedar is a native species of hard timber. In order to avoid over-harvesting of leaves from the young trees in the Agua Salud experimental plots (planted in 2008), leaves were obtained from a plantation of native species trees in the Soberania National Forest, 20 km Northwest of Panama City, Panama.

These plantations were created and maintained by PRORENA (the Native Species Reforestation Project), an organization associated with STRI's Center for Tropical Forest Science.

Treehole communities in this Central Panamanian region have been studied extensively. They can harbor multi-trophic level communities, including predatory mosquitoes and odonates (Yanoviak 2001a). Species richness and abundance of macroinvertebrates in these particular communities are impacted by the quantity of leaf litter as well as species specific quality of leaves (e.g. leaf toughness and C:N ratios) (Yanoviak 1999; 2001b). The system also allows for the use of artificial analogs for experimental manipulations. Artificial tree holes are highly replicable, easily sampled, and contain communities similar to those in natural holes; as such, they are excellent experimental units for research into effects of tropical tree diversity (Yanoviak & Fincke 2005).

*Experimental Design*

The experiment was located at STRI facilities in Gamboa, Panama (9°7′17″ N,79°42′11″W) in a grassy area near Experimental Pond between 12 July to 11 August of 2011. The field is partially shaded by large deciduous trees and is adjacent to secondary forest and riparian vegetation. We used a partial factorial litter mixing combination of Cocobolo, Spiny Cedar, and Teak that resulted in seven treatments: Co, Sp, Te, CoSp, CoTe, SpTe, CoSpTe (Table 1). We replicated each of the seven treatments nine times (63 cups) and arranged them in the study site in two rows with one meter between rows and 1 meter separating each artificial tree hole within a row. We randomly assigned treatments to locations within the study area. We created each artificial tree-hole by melting a 4 mm diameter hole through the side of a black, high-density plastic cup (Creative Converting™ Black Velvet 16 oz. cups; 475 ml; 12 cm height,

8.5cm and 5.6 cm diameters) using the tip of a soldering iron. The hole was located 2.5 cm from the top of the cup resulting in 360 ml of water and a surface area of 47.8cm$^2$ when full to the hole.

Black colored cups are recommended to mimic small treeholes (Yanoviak & Fincke 2005). The hole provided a place for water to escape and also made sure that a decent lip rose above the water surface and provided shelter for ovipositing mosquitoes. Insects are less likely to oviposit when the water surface is totally exposed (Correspondence with S. Yanoviak). Cups were then attached to the middle of bamboo stakes using duct tape and placed in the soil so that the bottom of the cup rested on the ground. Bamboo stakes were approximately 40 cm long and 1 cm in diameter and were obtained from nearby bamboo forests. Cups were then filled with leaf litter and water. In order to provide an oviposition surface, we placed a wooden plant label of 15cm x 2cm x 0.2cm into each cup so that a small amount of wood emerged from the top of the cup. At the start the experiment we filled each cup with a mixture of filtered (63µm mesh) rain water and tapwater.

*Leaf Litter Collection*

Fresh leaves are a principal component in tropical leaf litter and the bacterial colonization of tropical leaves occurs prior to abscission (Stout 1980); therefore, we collected fresh individual leaves directly from trees haphazardly chosen in the plantation on July 3 (Co, Sp) and July 9 (Te). We attempted to collect leaves as evenly as possible from at least 6 trees, sometimes as many as 12 (this depended on species). Leaves were brought back to the lab where we standardized leaf area and shape by cutting 2cm by 2cm square leaf fragments from each leaf. Leaf area is associated with decomposition rates and leaf size and shape may be an important

physical difference between the tree species (Cornejo et al. 1994). While this erases certain differences between leaf litter types, small fragments were needed to manipulate mass. We placed all leaf litter within the drying oven at 60 C within 24 hours of it being collected and cut. Leaf litter remained in the drying oven until it was needed for a leaf litter addition at which point it was weighed and counted. Each cup received the same amount of leaf litter based on dry mass at any given leaf litter addition. Leaf litter additions were added regularly for each experiment in order to maintain leaf litter characteristics. We weighed leaf litter to within 0.01g of the predetermined mass and counted the number of fragments contributing to this mass for each cup at each time point. We estimated surface area as the number of leaf fragments placed in a cup over the course of the study multiplied by 4 $cm^2$ (2cm x 2cm fragments). Each cup received 2.4 g total mass of leaf litter by the end of the study regardless of species combination and each species of leaf litter contributed in equal proportion for mixed leaf litter treatments. The initial leaf litter addition was 1.2 g while the subsequent two additions were 0.6 g of dry mass. Leaf litter additions occurred on July 12, July 20, and July 31. We also dried 10 fragments of each species to determine proportion dry mass and water loss. Leaf litter C:N ratios were obtained from unpublished data as an additional measure of leaf quality (D. Craven unpublished data; Kominoski et al. 2009).

The experiment began when water was added to each cup containing leaf litter on July 12, 2011. For the first 10 days of the experiment (July 13-22), we checked every cup for oviposition and counted individual eggs and egg rafts from mosquitoes. We sampled the cups for invertebrates July 19-20 (cup order 1-72), July 28-30 (cup order 72-1), and Aug 7-8 (cup order 72-1). During sampling we poured the entire contents of a cup into a white tray and removed all the leaf litter fragments from the cup. We visually identified and counted all macroinvertebrates.

We counted and identified later instar mosquito larvae to similar larval morphospecies and counted all neonates. Larval morphospecies were a general identification for abundance; identification of mosquitoes was based on adults reared in the lab (see section *Invertebrate Identification*).

We kept all pupae as their roles in the community are strictly as prey items; however, we only removed between 1 to 3 larval individuals of a morphospecies from any cup for rearing and identification purposes. We brought invertebrates back to the lab in 400 ml whirlpacks in a cooler. Prey and predatory species were not stored in the same bags so as to prevent loss of individuals. While non-randomly removing invertebrates from small communities for rearing and identification may change community dynamics (Townsend 1989), care was taken not to remove all individuals of a species from the community (except in the case of obtaining necessary vouchers). These communities typically have such high turnover levels, that removing a few individuals should not severely change community dynamics and previous studies have used similar methods (Yanoviak 1999). We added all remaining invertebrates, water, and leaf litter back to the cup following sampling.

*Leaf Litter Mass Loss and Water Quality*

When we terminated the experiment on August 11, we brought all contents in each cup back to the lab. We initially filtered the contents through 1 mm mesh veil and set aside all water. We then rinsed all solid materials with clean, aged tap water and collected leaf fragments by hand to ensure that no larvae accompanied the solid litter. Within 12 hours of the experiment termination in the field, we had removed and processed all leaf litter. Upon removal, we set the leaf litter from each cup in an air-conditioned laboratory for 3 days to assist in the drying

process. On August 15 we placed the leaf litter in a drying oven at 60°C where it remained until August 20 when we weighed the leaf litter from each cup.

The water was subsequently poured through 119 µm mesh, and then vacuum filtered using a pump driven filtration apparatus through 1.6 µm Whatman grade GF/A ashless filter paper. Approximately 45 ml of filtered water was then poured into 50 ml Corning centrifuge tubes and frozen for shipment back to the United States. Virginia Commonwealth University's Environmental Analysis Laboratory (EAL) thawed and analyzed each sample for dissolved organic carbon and nitrogen from September 19-22, 2011. The EAL determined dissolved organic carbon (DOC), total nitrogen (TN), the mono nitrogen oxides, $NO_2$ and $NO_3$ ($NO_x$), and ammonia/ammonium ($NH_3$ and $NH_4^+$). All measurements were in mg/L. DOC was measured using the SM 5301B method on a Schimadzu TOC-5000 analyzer. All nitrogen measurements were conducted using a Skalar SanPlus Segmented Flow Analyzer. Total nitrogen was measured using the DeLeia method. Inorganic forms $NO_x$ and $NH_3$+$NH_4^+$ were measured using the SM 4500-NO3F and EPA 350.1 methods, respectively. The difference between TN and the inorganic components, $NO_x$ and $NH_3$, was used to represent the dissolved organic nitrogen (DON). We then calculated C:N ratios using DOC and DON. See the VCU EAL standard operating procedure (2011) for further information.

*Invertebrate Identification*

In the lab, we placed similar species in plastic cups with a few centimeters of water. A wooden craft stick was included in each cup to break the water surface and provide a perch for emerging insects and 0.25 mm mesh netting secured with rubber bands prevented escape (Correspondence with S. Yanoviak). Larvae were fed with freshwater fish food flakes and

allowed to grow to adulthood. Cups were checked at least once a day for emerged adults. If adults were present, the water was drained from the cups and adults were allowed to dry and harden in the cups for 24 hours. At that point they were killed by freezing (12-24 hrs), and then identified. All adult insect vouchers were mounted by pointing using card stock and white glue with number 3 pins. Mosquitoes were oriented on the point with the right side of the thorax glued to the point. Larval vouchers were preserved using 95% ethanol. Voucher specimens were deposited with STRI and the Invertebrate Museum of the University of Panamá. Invertebrates were identified to the lowest possible level using published keys and consultation with regional expert (S. Yanoviak; Clark-Gil & Darsie 1983; Stehr 1987, 1991).

*Statistical Analyses*

We used one-way ANOVA models to test the effects of leaf litter treatment on the response variables. Responses were examined for normality and heteroscedasticity using Shapiro-Wilkes and Levene's test, respectively. Significance in either test resulted in transformation of responses to best fit assumptions. Leaf litter loss and the natural log of C:N ratios were analyzed using standard linear models. Overall invertebrate abundance and specific abundances of three species were transformed using square-root transformation to better fit a normal distribution (specifically sqrt(x+0.5)). For count-based responses we explored the use of generalized linear models with alternative distributions; however, due to over-dispersion we opted for linear models using transformed variables.

In order to investigate the overall effects of leaf litter richness on the responses, we treated leaf litter richness (1, 2, 3) as a continuous variable and fit regressions. While leaf litter richness could be considered a categorical variable the values are at equal intervals over a small

range and treating them as a continuous predictor would be valid in this case. We used linear regression to test the effects of total leaf surface area on litter mass loss/breakdown. We also used correlations to test for relationships between total macroinvertebrate abundance and the dissolved carbon and nitrogen responses (C:N, DOC, and DON).

To determine whether effects of richness were non-additive, we created contrasts between polycultures and their respective monoculture constituents using linear hypothesis tests. These tests compared the observed response from a polyculture to an expected value of the response variable based on the proportional contributions of the observed response in the constituent monocultures given no interactive effects (Chapman et al 1988). As all of my polyculture treatments have equal mass proportions from the contributing species, an additive response would result in the observed response being equivalent to the average of the monoculture observed responses in that mixture. A non-additive response resulted when the linear hypothesis test showed the difference between the polyculture observed response and the additive prediction was not equal to zero (e.g. CoSp - (Co+Sp)/2 $\neq$ 0). In instances where we detected significant non-additivity in a polyculture we used the $D_{max}$ method to attempt to tease apart complementarity and selection effects (Loreau 1998; Wodjak & Mittelbach 2007):

$$D_{\max} = \frac{O_T - \max(M_i)}{\max(M_i)} \qquad \text{Eq. 1}$$

where $O_T$ is the observed total yield of the mixture and max($M_i$) is the maximum observed yield of a species in monoculture from that mixture. If $D_{max} \leq 0$, we are unable to determine whether complementarity and/or selection effects are causing non-additivity. If $D_{max} > 0$, the polyculture is confirmed to be overyielding and therefore sampling or selection effects are eliminated. If we

expect antagonistic interactions (i.e. the polyculture has a lower response than the monocultures, e.g. C:N ratios), this equation is reformatted as:

$$D_{max} = \frac{\min(M_i) - O_T}{\min(M_i)} \quad \text{Eq. 2}$$

where min($M_i$) denotes the monoculture component with the lowest yield. $D_{max} > 0$ still excludes sampling effects. We also compared polyculture responses to the best performing monoculture yield.

All statistical analyses were conducted using R statistical package 2.13.1(The R Foundation for Statistical Computing 2011).

## RESULTS

*Leaf Litter Mass Loss*

Litter mass lost varied among leaf litter treatments ($F_{6, 56}$ = 43.2, P < 0.0001). The singular species treatments, Co, Sp, and Te, all differed from one another with Sp losing 33% more mass than Te (P < 0.0001; Fig 1a) and 89% more than Co (P < 0.0001;Fig 1a). The Co leaf litter lost less mass than every other leaf litter treatment (all pairwise comparisons P < 0.05; Fig 1a). The next highest loss was from the CoTe mixture which was different from all other treatments except for Te (CoTe – Sp: P < 0.0001; CoTe - CoSp: P < 0.0001; CoTe - Co: P = 0.015; CoTe - SpTe: P < 0.0001; CoTe - CoSpTe: P < 0.0001). Te had similar losses to CoSp and CoSpTe but lost 19% less than SpTe (P = 0.0001); however, CoSp and CoSpTe did not differ from SpTe. SpTe and Sp lost the most mass over the course of the study (Fig 1a). Sp lost 20% more than CoSp and CoSpTe and 54% more than CoTe (Sp – CoSp: P=0.0002; Sp – CoSpTe: P=0.0002; Sp – CoTe: P<0.0001). Leaf litter mass loss was not related with leaf litter richness in the treatment ($R^2$=0.03, d.f.=61, P=0.21; Fig 1b). Linear hypothesis tests showed there was marginally significant deviation from additive predictions based on the constituent monocultures for the three species treatment; CoSpTe lost 9.4% more litter mass than predicted based on its components (CoSpTe: $F_{1, 56}$ = 3.5, P=0.066). $D_{max}$ for CoSpTe was -0.17. None of the other polycultures differed from predictions (CoSp: $F_{1, 56}$ = 2.92, P =0.09; CoTe: $F_{1, 56}$ = 0.32, P =0.57; SpTe: $F_{1, 56}$ = 0.87, P = 0.35; Fig 1a).

Leaf litter loss was strongly predicted by total leaf surface area ($p < 0.0001$; Fig 2). Surface area explained 75% of the variation in leaf litter loss. A 100 $cm^2$ increase in surface area resulted in a 0.2g increase in litter loss.

*C:N Ratios*

Ratios of dissolved organic Carbon and Nitrogen varied among leaf litter treatments ($F_{6, 56} = 11.8$, $P < 0.0001$). Specifically, Co and Sp had similar, higher ratios than all other treatments ranging from 53% higher than CoTe to 99% higher than Te (all pairwise comparisons $P < 0.05$). The ratios of the other five treatments did not statistically differ from one another (Fig 1c). C:N ratios were negatively related with leaf litter richness in the treatment ($\ln(y) = -0.26x + 3.0$, d.f.=61, $P<0.001$; Fig 1d) and leaf litter richness explained 21% of the variation in C:N ratios. As litter richness increased, there was a corresponding decrease in C:N ratios; however, as the line is based on log-normalized C:N ratios, this decrease becomes smaller with each subsequent higher level of richness (Fig.1d). Litter types combined synergistically to influence nutrient quality. Polycultures often had higher nutrient quality (i.e. low C:N) than predicted by their respective monocultures (Fig 1c). The CoSp and CoSpTe polycultures had 41% and 38% lower C:N ratios, respectively, than predicted from their monocultures (CoSp: $F_{1, 56} = 10.2$, $P=0.002$; CoSpTe: $F_{1, 56} = 10.9$, $P=0.002$; Fig 1c). $D_{max}$ values for CoSp and CoSpTe were 0.36 and -0.004, respectively. However, the 33% and 13% lower than predicted respective ratios of SpTe and CoTe were not statistically significant (SpTe: $F_{1, 56} = 2.7$, $P = 0.11$; CoTe: $F_{1, 56} = 0.47$, $P =0.50$; Fig 1c).

*Invertebrate Diversity*

We did not analyze statistical differences in species richness as species richness was overall much lower than expected, so we summarize major trends here. In the final timepoint, highest mean species richness was in the SpTe treatment (2.3 ± 1.12 species per cup; ±SD; Table 2). CoSp and CoTe, each had 2 species on average (±0.71 and 1.00 respectively; Table 2). CoSpTe had slightly fewer species at 1.9 species per cup (±0.78) and Co and Sp each had the same average of 1.7 species per cup (±0.71 and 0.50, respectively). Te had the fewest species with 1.3 per cup (±0.50). Only one cup in the entire study contained more than three species (an SpTe cup in the 3rd time point had 4 species). We identified a total of thirteen different invertebrate morphospecies over the entire study period. Of these, eight were mosquito morphospecies from four different genera including the invasive Asian Tiger mosquito, *Aedes albopictus* (Table 2). Single individuals of a Hydrophilidae beetle (<3mm in size), *Dero* sp. annelid, and a copepod were found during the study; the latter two identified during time point 1. The mosquito species *Limatus durhamii* and *L. asulleptus* (addressed collectively as *Limatus* spp.), *Trichoprosopon digitatum*, and *Culex* sp. 1 were the most abundant of all taxa found. Therefore we concentrate on the abundances of these mosquitoes.

*Total Invertebrate Abundance*

Total macroinvertebrate abundance varied among leaf litter treatments ($F_{6, 56} = 2.33$, $P = 0.044$; Fig 3a); however, post-hoc comparisons showed only one significant difference in the treatment groups. Cups with Te held on average the fewest number of individual macroinvertebrates while CoSpTe had the greatest mean abundance containing approximately 220% more individuals than Te ($P < 0.03$; Fig 3a). Transformed abundance was significantly

related with leaf litter richness in the treatment (sqrt(y+0.5)= 1.2x+5.3, d.f.=61, P=0.02; Fig 3b). Leaf litter richness explained 8% of the variation in in macroinvertebrate abundance; as litter richness increases, there is a corresponding increase in abundance (Fig.2b). Linear hypothesis tests showed some marginally significant evidence of synergistic behavior with respect to abundances (Fig 3a). The SpTe and CoSpTe polycultures had 114% and 72% greater abundances, respectively, than predicted from their monocultures with $D_{max}$ values of 0.65 and 0.18 (SpTe: $F_{1, 56}$ = 3.6, P=0.06; CoSpTe: $F_{1, 56}$ = 3.4, P=0.07; Fig 3a). The observed abundances in CoSp and CoTe were not statistically different from the additive predictions (CoSp: $F_{1, 56}$ = 1.09, P = 0.3; CoTe: $F_{1, 56}$ = 0.19, P =0.66; Fig 3a).

Total abundance was not correlated with dissolved C:N or DOC (p=0.27 and 0.07, respectively; Fig 4, 5). Total abundance was negatively related with dissolved organic nitrogen (p=0.001, $R^2$=0.16; Fig 6). Patterns were similar with total nitrogen and when C:N ratios incorporated total nitrogen instead of solely organic nitrogen.

*Abundance of* Limatus *spp.*

The abundance of larval *Limatus* spp. varied among leaf litter treatments ($F_{6, 56}$ = 3.9, P =0.0025; Fig 3c). Only Sp had different average abundance from the other treatments. *Limatus* individuals were less abundant in Sp than in cups containing Co, CoTe, SpTe, and CoSpTe (Sp – Co: P = 0.018; Sp - CoTe: P = 0.039; Sp – SpTe: P = 0.024; Sp - CoSpTe: P = 0.026; Fig 3c). There was also a marginally significant difference between Sp and CoSp (P = 0.068). All of these treatments had *Limatus* abundances two orders of magnitude greater than the Sp treatment. Abundance of *Limatus* was related with leaf litter richness (sqrt(y+0.5)= 1.3x+1.6, d.f.=61, P=0.008; Fig 3d). Leaf litter richness explained 11% of the variation in *Limatus* abundance. As

litter richness increases, there is a corresponding increase in number of *Limatus* larvae (Fig.2d). Litter types sometimes combined synergistically with respect to *Limatus* abundance resulting in abundances greater than additive predictions (Fig 3c). SpTe had an abundance of *Limatus* larvae approximately 1000% greater than predicted based on its constituent monocultures and $D_{max}$ equaled 5.3 (SpTe: $F_{1, 56} = 7.6$, P=0.0077; Fig 3c). The observed abundances in CoSp, CoTe, and CoSpTe were not different from the additive predictions (CoSp: $F_{1, 56} = 0.27$, P = 0.6; CoTe: $F_{1, 56} = 0.06$, P =0.8; CoSpTe: : $F_{1, 56} = 1.8$, P =0.18 Fig 3c).

*Abundance of* Trichoprosopon digitatum

The abundance of larval *T. digitatum* varied among leaf litter treatments ($F_{6, 56} = 3.09$, P =0.01; Fig 3e). *Trichoprosopon* were found in higher abundances in cups containing Sp than in any other treatment (Sp – SpTe: P=0.07; all other pairwise comparisons < 0.05). There were no differences in abundance of *T. digitatum* between any of the other leaf litter types (Fig. 2e). Abundance of *T. digitatum* was not related with leaf litter richness (sqrt(y+0.5)= -0.4x+2.6, $R^2$=0.03, d.f.=61, P=0.18; Fig 3f); however, there was some marginal evidence of *T. digitatum* abundance behaving antagonistically in polyculture (Fig 3e). CoSp had an abundance of *T. digitatum* larvae approximately 82% less than predicted and a $D_{max}$ of 0.38 (CoSp: $F_{1, 56} = 3.4$ P=0.069; Fig 3c). The observed abundances in CoTe, SpTe, and CoSpTe were not statistically different from the additive predictions (CoTe: $F_{1, 56} = 0.75$, P = 0.39; SpTe: $F_{1, 56} = 1.25$, P =0.27; CoSpTe: : $F_{1, 56} = 0.25$, P =0.62 Fig 3c).

*Abundance of* Culex *sp.*

The abundance of larval *Culex* sp. did not differ among leaf litter treatments ($F_{6, 56}$ = 0.98, P =0.45; Fig 3g). *Culex* abundance was not related to leaf litter richness (y= 0.77x+0.59, $R^2$=0.04, d.f.=61, P=0.10; Fig 3h). *Culex* abundance in polycultures did not deviate from additive predictions (CoSp: $F_{1, 56}$ = 0.98, P = 0.33; CoTe: $F_{1, 56}$ = 0.22, P =0.64; SpTe: $F_{1, 56}$ = 1.1, P =0.29 CoSpTe: : $F_{1, 56}$ = 3.1, P =0.08; Fig 3g).

# DISCUSSION

Understanding how terrestrial habitats impact the aquatic communities embedded within them is a fundamental question in ecology with important conservation and management implications as natural forest continues to be converted into less diverse habitat at an alarming rate. The results of this study show that species identity and richness of leaf litter from a set of economically important trees have significant effects on resources and mosquito abundances within aquatic tree hole communities. Both leaf type and richness affected nutrient quality and breakdown as well as total invertebrate abundance and the abundances of the most common taxa. In general, more diverse litter treatments had higher quality nutrients and greater mosquito production.

## *Litter Species Identity*

All three single species treatments were different from each other in leaf litter loss and Teak treatments had lower dissolved C:N ratios than Cocobolo and Spiny Cedar. We hypothesized that tree species known to have high quality leaf litter (low C:N ratios) would have lower dissolved C:N ratios in water and more leaf litter mass lost over the course of the study. Previous measurements of leaf C:N ratios from these trees showed that Cocobolo leaves have lower C:N ratios, likely because this species is the sole nitrogen fixer in our chosen species. Based on this we would have expected Cocobolo to have lower dissolved C:N ratios and more mass loss. This was not the pattern we observed. Cocobolo lost the least mass, followed by Teak

and then Spiny Cedar. Cocobolo was a much tougher leaf than Spiny Cedar or Teak and, following the study, leaf fragments of Cocobolo were structurally intact; conversely, Spiny Cedar and Teak leaves had fallen apart leaving very little structure left. While Cocobolo's leaves are rich in nitrogen, they leach very little of that nitrogen into the water and do not break down readily. The low dissolved C:N ratio of Teak was unexpected considering its high initial C:N ratios (Table 1). Typically, filtering for DOC and DON measurements uses a 0.4 to 0.7 µm filter and some studies recommend as low as 0.1 µm (Chow et al. 2005); however, we used a 1.6 µm filter due to material availability. This could result in higher observed levels of leached DOC and DON than actually exist as some POC (particulate organic carbon) or bacteria could have been included in the filtrate. However, inadequate filtration would have affected all treatments.

The lower C:N ratio in Teak was driven by lower levels of carbon rather than higher levels of nitrogen (Table 3). In fact, Teak had lower dissolved nitrogen than all treatments, excluding Cocobolo. Dissolved nitrogen is an important part of aquatic communities and may be taken up directly by microbiota in the system (Berman & Bronk 2003). These differences could be due to actual differences in leaching between the species or from biological interactions such as the presence of particular consumers resulting in different breakdown.

Some of these resource differences carried through to community responses. Lower dissolved C:N ratios are considered higher quality, as nitrogen is typically limited in aquatic systems and can lead to increased microbial production supporting higher consumers (Verdonschot et al 2008) including mosquitoes (Walker et al. 1991). Even though Teak cups had the lowest aquatic C:N ratios, they supported the lowest total abundance of invertebrates among the three species. Walker et al. (1997) showed greater survival and growth of mosquitoes in containers with lower dissolved C:N, so something else may be driving the low abundances in

Teak such as unknown leached compounds. Teak wood is known for being highly resistant to decay and attack by termites (Nichols et al. 2002) and some of these qualities may also be present in leaves. In addition, as mentioned earlier, the low C:N ratios in Teak were driven by lower carbon rather than higher nitrogen. We believed that C:N ratios in the water would note high quality habitat; however, our correlations provided support that aquatic dissolved nitrogen may be a better indicator than C:N or DOC for a better resource supported community.

We had relatively low invertebrate species richness in all of our cups. This could be due to several reasons. The main one is most likely that the communities were very young. Artificial treeholes especially need time to develop into mature treehole communities. However, as container habitats, or newly filled treeholes, this system is still highly relevant. Many mosquito species are early colonizers, both to avoid competition and predation. Since they impact later colonizers through priority effects, effects of litter diversity on these early colonizers can be important for later community development (Sunahara & Mogi 2002). There is also a possibility that the regional species pool was low. While the common garden was up against riparian vegetation and a stream, it was close to the human community in Gamboa, Panama where they do fog for mosquitoes occasionally. Aerial pesticide application can have unexpected and synergistic effects on aquatic communities around urban settings (Weston et al. 2006); however, we do not concentrate on those in this study. Recent efforts in Gamboa since the conclusion of the study to reduce container habitats for mosquitoes may have impacts on future studies in the region.

While Cocobolo and Spiny Cedar had very different losses in leaf litter, there was no difference in total abundance between the two types. But when we examine the abundance of individual taxa, interesting differences emerge. *Limatus* spp. were highly abundant in Cocobolo,

but rare in Spiny Cedar. Conversely, *T. digitatum* was highly abundant in Spiny Cedar and not very abundant in Cocobolo or Teak. We address this pattern later in discussion. The presence of *Culex* sp. 1 was unpredictable; as *Culex* oviposit eggs in large rafts, many larvae will enter a cup at the same time but rafts were not in all cups of a treatment. This results in high maximum abundances but lower, highly variable means (Table 2; Figure 5g). The resulting variance makes it difficult to detect patterns in *Culex* abundance. Of the three common taxa we addressed, *Culex* spp. seems to disregard litter type as important while *Limatus* spp. and *T. digitatum* favor different leaf litter species.

*Litter Richness and Additivity*

In general, significant effects of leaf litter richness arose from synergistic effects of polycultures on nutrients and consumers. However, if leaf litter richness did not have a significant effect it did not necessarily mean all mixtures combined additively. Additivity implies that mixtures behaved in a linear fashion with respect to their single species components, or in other words the combined response can be predicted from the response of each species when alone. We utilized a substitutive design so the response from a two species mixture is expected to be the average of the observed responses in the monocultures (i.e. CoSp = (Co+Sp)/2). Our substitutive design controls for effects of mass: since our treatments had equal total mass, higher responses in polycultures do not simply reflect differences in total resource input to the system. A non-additive response reflects an interaction between leaf litter types. Loreau and Hector (2001) suggest two effects that can lead to non-additivity: complementarity effects and selection effects (see Fox 2005 for a tripartite separation of effects).

Complementarity effects are caused when polycultures perform better at the community level than expected from their performance at monoculture. They are explained by mechanisms such as complementary resource use (niche differentiation) or facilitation. Selection effects occur when the performance of a polyculture is driven by a single dominant species with respect to its relative abundance in the polyculture (Loreau & Hector 2001; see Huston 1997 for the "sampling effect"). Selection and complementarity effects each result in non-additivity and it is important to attempt to separate them; however, in order to do so using the Loreau and Hector (2001) method requires knowing the relative abundances of the species in polyculture at the end of the experiment. For producer studies, this is logical as plants will continue to grow and create new biomass after planting. Detrital studies are different in this respect although some recent studies take into consideration different litter composition at the end of an experiment (Treplin & Zimmer 2012). We were unable to assess this in our study in this way as we could not separate Spiny Cedar and Teak leaves at the end of an experiment when they were in mixtures together. Therefore, we discuss possible mechanisms for patterns we saw and in instances when we found evidence for non-additivity we conservatively separate complementarity and selection effects by comparing the response in polycultures to that of the highest yielding component monoculture (Wodjak & Mittelbach 2007).

In general, increasing litter richness trended towards improved quality/performance. Litter mass loss trended in the direction of a positive effect of litter richness, but while all mixtures lost more than expected, only for the three species polyculture were the expected and observed even marginally different. While this may be evidence for non-additivity we are not able to determine whether it was due to complementarity or selection effects as the $D_{max}$ was less than zero. Spiny Cedar could be dominating and driving this effect in leaf litter breakdown with

no change in Cocobolo or Teak breakdown or there could be interactions between leaf litter types such as nutrient facilitation. Swan and Palmer (2004) similarly found no significant overall effect of litter richness but found strong evidence for non-additivity in most combinations in their temperate stream study (Swan & Palmer 2004). While they had a greater number of leaf litter types (five), and therefore more possible comparisons, lentic systems may simply be more prone to non-additivity. Leaf litter breakdown in streams occurs at a faster rate than in lakes and wetlands due to flowing water causing mechanical breakdown (e.g. leaves thrown against rocks) and by introducing new oxygen and detritivores (Webster & Benfield 1986). We controlled for leaf mass among treatments but since the leaf species differ in mass per surface area, surface area varied by treatment and in a completely additive way. Litter breakdown was heavily dependent on surface area in the cups and this relationship was not decoupled in higher polycultures which would explain our lack of non-additivity in leaf litter loss.

C:N ratios decreased with litter richness (i.e. quality increased as we predicted) and this relationship was heavily supported by non-additivity in the mixtures. Across responses, the strongest evidence for non-additive combination was between Cocobolo and Spiny Cedar with respect to C:N ratios. As single species treatments, the C:N ratios of Co and Sp were both elevated and very similar (Fig 1c). When in polyculture, the C:N ratios were 38% lower than predicted and $D_{max}$ for CoSp was well above zero. This rules out selection and sampling effects as neither Cocobolo nor Spiny Cedar had C:N ratios near this level when in monoculture. These results are particularly interesting as the combination of these two species did not lead to non-additive effects on mass loss. The decreased C:N ratio was driven by a doubling in the dissolved organic nitrogen content but DOC that was similar to that in the monocultures (Table 3). In most cases nitrogen composes less than 1% of leaves by mass, meaning a 2-fold increase in leached

nitrogen is likely to have little impact on the overall mass of the leaf (McClaugherty et al. 1989). Leaching occurs rapidly in aquatic habitats and leaf litter is left as a relatively nutrient poor substrate; bacteria in this system may compensate for this by assimilating dissolved organic matter (Treplin & Zimmer 2012). Cocobolo is a nitrogen fixer but has poor breakdown and small surface area (Table 3); Spiny Cedar had higher surface area and high mass loss. While we cannot determine the mechanism without additional experiments, we hypothesize that breakdown of Spiny Cedar leaves may have facilitated increased microbial colonization of Cocobolo and contributed to release of nutrients from Cocobolo. CoSpTe also showed evidence of a non-additive response to mixing. $D_{max}$ was less than zero due to Teak's low C:N ratio. As such we cannot determine if non-additivity was due to complementarity, selection effects, or some combination of both. But, considering the presence of Cocobolo and Spiny Cedar in this treatment, complementarity is likely to play some part.

Rapid leaching and decomposition is also prevalent in fresh leaves. While we used fresh leaves (based on the literature and logistical reasons), it is important to note that fresh and senescent leaves behave differently during the leaching and breakdown processes. During abscission, trees remove important nutrients from leaves that were still present in our leaf litter. As such, decomposition moves more rapidly, leaves contain more nitrogen, and the overall quality of the resources is higher (Fonte & Schowalter 2004). If we had used senescent leaves, we may have seen lower quality C:N ratios, slower breakdown, and lower invertebrate abundances. The patterns in non-additivity (especially for breakdown) may have also been different as studies in temperate systems typically use senescent autumn leaf fall in the decomposition studies (Swan & Palmer 2004).

Total abundance, *Limatus* sp. abundance and *Culex* sp. abundance (trend but not significant) all increased with the number of litter species present in a cup. Interestingly, *T. digitatum* abundance trended in the other direction as abundance decreased with higher litter species richness. Patterns in total abundance across treatments did not follow those observed in either C:N ratios or leaf litter breakdown as we hypothesized, however marginal non-additivity was still present. The strong non-additivity from the CoSp treatment seen in C:N ratios was non-existent in total abundance as the observed abundance was somewhat lower than expected. Instead, marginal non-additivity was observed in SpTe and CoSpTe. $D_{max}$ values for both were larger than 0 implying that selection effects were not likely; however, SpTe and CoSpTe were not statistically different from their highest performing monoculture components (Sp and Co respectively). Our calculated $D_{max}$ does not take into consideration variance in the response and as such, we are wary of disregarding selection effects.

Resources may not fully explain the patterns seen in total abundance across the treatments. These may be driven by patterns observed in individual taxa. The pattern describing the presence/absence of *Limatus* spp. larvae and *T. digitatum* larvae we mentioned earlier may contribute to those seen in total abundance. *Trichoprosopon digitatum* is a detritivore but is known to prey opportunistically on other larvae as well as cannibalize; however they do not consume larvae larger than themselves (Sherratt et al 1999, Church & Sherratt 1996). Large *T. digitatum* larvae also disregarded neonates when they reached a certain size (personal observation). *Trichoprosopon digitatum* was the only species found as a late instar in the first time point during which it was most abundant in Teak (see supplementary Figure 7). Their early colonization of cups may be to ensure that they are not consumed by conspecifics; the species is unique for its clutch guarding behavior and females will preferentially lay eggs in containers

with females currently guarding new clutches and avoid containers with later instar larvae (Sherratt & Church 1994). In addition, previous studies have found that it colonizes containers with high food levels – its preference for Spiny Cedar in our study could be due to females recognizing the highly labile carbon available in this treatment given its rapid breakdown (Table 3; Sherratt & Church 1994). As *Limatus* larvae (and the adults) are smaller than *Trichoprosopon*, we hypothesize *Trichoprososprон* larvae in the Spiny Cedar treatment are either consuming the smaller *Limatus* larvae before they become late instars or out-competing them for resources. An alternative hypothesis is that *Limatus* females are avoiding *Trichoprosopon* larvae and therefore not ovipositing in the Spiny Cedar cups to the same degree. A recent meta-analysis showed that certain mosquitoes can and will avoid ovipositing in larval habitats containing predators (Vonesh & Blaustein 2010); however, our observational data on oviposition suggest the predation hypothesis is a better explanation than habitat avoidance by *Limatus*. During the first sampling point (July 19 and 20) and the two days following it, the average number of *Limatus* eggs found on cups containing late instar *Trichoprosopon* was 6.2±7.32 (±SD, n=56) while the average number of *Limatus* eggs across all cups during those days was 9.8±12.47 (n=252). We recommend future investigations assessing the interaction between *Limatus* and *Trichoprsopon* which include oviposition trials with *Limatus* across cups with or without *Trichopropon* as well as survival and growth trials for *Limatus* with or without the exclusion of *Trichoprosopon.*

*Aedes aegypti,* the invasive yellow fever mosquito native to Africa, is a common container inhabitant in Panama City, Panama (Perich et al. 2000). However, we did not identify a single individual from this highly conspicuous species. In its place we found *Aedes albopictus,* the highly invasive mosquito commonly known as the Tiger mosquito native to Southeast Asia which has quickly spread world-wide (Knudsen 1995). This was not the first discovery of the

species in the region; the species was identified in Panama for the first time in 2002 (personal correspondence with Jim Pecor of the Walter Reed Biosystematics Unit). However, of import is its apparent displacement of *A. aegypti*. Previous studies have shown *A. albopictus* to be the clear superior larval competitor in varying environmental conditions when placed in direct competition with *A. aegypti* (Braks et al. 2004). Considering their similar life histories and characteristics as disease vectors, the replacement of *A. aegypti* with *A. albopictus* may not have a strong effect on community dynamics or human health. *Aedes albopictus* is typically a container-breeding mosquito that favors areas impacted by anthropogenic processes, rather than a forest tree hole inhabitant (Knudsen 1995). Altered landscapes like those following timber harvest may be prime habitats for disease vectors like *A. albopictus*.

Given our results, both leaf litter identity and diversity appear to be important determinants in patterns of litter breakdown, aquatic nutrients, and invertebrate abundance in tree hole communities. Given the presence of disease vectors and dynamics between mosquito species, understanding the effects of tree diversity on these systems is important. Here we examined the effects of richness and identity of litter from three timber species, including an exotic timber species. The position and characteristics of these artificial tree holes may emulate stumps created during selected harvesting of timber in forest management practices. Teak is of high importance due to its increasing prevalence in plantations worldwide and is typically planted in monoculture even though its native forests in Asia are mixed-species assemblages (FAO Teak assessment). The increasing consideration of forestry management practices beyond simply production will improve overall ecosystem services and can contribute to ecological theory beyond forestry plantations. A landscape experimental manipulation such as the Agua Salud project provides a prime base for taking these diversity questions to the next level. While

understanding the effects of litter mixing is important, future studies should investigate the effects of diversity on the system when the aquatic communities are placed in the context of the actual terrestrial landscape and not just a common garden.

## ACKNOWLEDGMENTS

We thank the Autoridad Nacional del Ambiente de Panamá for permission to conduct this research in Panama (Permiso No.SC/AP-2-11) and the Smithsonian Tropical Research Institute for use of their facilities and logistical support. We send special thanks to the technicians with the Agua Salud Project and PRORENA plantations. We also would like to thank Dr. Steve Yanoviak and Dr. Jefferson Hall for sharing their expertise about tree hole communities and forest diversity management, respectively. We also thank Dr. Rima Franklin and Dr. Kevin Caillouet for insightful comments and helpful critiques on the study design and manuscript. Current and former Vonesh lab members also provided helpful feedback for early drafts. The Gamboa, Panama "Fear and Death" group also deserves thanks for assistance and logistical support in the field.

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## Appendix

**Table 1.** Species specific traits of trees and leaf litter. Carbon to nitrogen ratios of leaves obtained courtesy of Dylan Craven; leaves were taken from 10-12 trees in the PRORENA plantations in Soberania in 2005 (D. Craven, unpublished data). Values presented are means ± SE. We were unable to obtain the SE for Teak. Percent mass loss due to drying calculated from 10 leaf fragments of each species prior to beginning the study.

| | Cocobolo | Spiny Cedar | Teak |
|---|---|---|---|
| C:N ratio in leaves* | 18.6±1.35 | 20.9±1.52 | 25.5 |
| Mass lost to drying (%) | 66±3.2 | 80±2.1 | 69±5.8 |
| Native? | Y | Y | N |
| Nitrogen fixer? | Y | N | N |

**Table 2.** Invertebrate taxa found in each treatment. Values are maximum abundances found in that treatment for a particular species across all three time points. Stage is the life stage of the invertebrate found in the cup. Minimum abundance of each species was zero in all treatments. Mean species richness is the average number of species (±SD) in a treatment at each of the three time points.

| Taxon | Stage | Co | Sp | Te | CoSp | CoTe | SpTe | CoSpTe |
|---|---|---|---|---|---|---|---|---|
| Diptera: Culicidae | | | | | | | | |
| *Limatus durhamii* & *L. asulleptus* | Larva | 116 | 17 | 14 | 163 | 80 | 93 | 82 |
| *Trichoprosopon digitatum* | Larva | 32 | 48 | 38 | 38 | 37 | 32 | 29 |
| *Aedes albopictus* | Larva | 6 | 0 | 0 | 3 | 0 | 2 | 2 |
| *Culex* sp. 1 | Larva | 93 | 84 | 75 | 71 | 101 | 105 | 88 |
| *Culex* sp. 2 | Larva | 0 | 0 | 0 | 0 | 23 | 0 | 0 |
| *Culex* sp. 3 | Larva | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Culex* sp. 4 | Larva | 0 | 0 | 0 | 0 | 0 | 63 | 0 |
| Diptera: Muscidae | Larva | 1 | 14 | 4 | 0 | 1 | 0 | 0 |
| Diptera: Psychodidae | Larva | 1 | 0 | 0 | 0 | 3 | 1 | 0 |
| Coleoptera: Hydrophilidae | Adult | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Annelidae: Naididae | | | | | | | | |
| *Dero* sp. | Adult | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Crustacea: Copepoda | Adult | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Mean Species Richness | | | | | | | | |
| Time Point1 | | 0.3 (0.71) | 0.3 (0.50) | 0.7 (0.71) | 0.2 (0.44) | 0.6 (0.73) | 0.3 (0.50) | 0 (0) |
| Time Point 2 | | 2.0 (1.00) | 1.0 (0.50) | 1.7 (0.87) | 2.0 (0.71) | 1.4 (0.53) | 1.9 (0.60) | 1.9 (0.78) |
| Time Point 3 | | 1.7 (0.71) | 1.7 (0.50) | 1.3 (0.50) | 2.0 (0.71) | 2.0 (1.00) | 2.3 (1.12) | 1.9 (0.78) |

**Table 3.** Leaf surface area ($cm^2$) and dissolved nutrients (mg/L) in each treatment. Values are means and SD. DOC = dissolved organic carbon; TN = total nitrogen; $NO_x$ = total amount of mono nitrogen oxides.; $NH_3$ = ammonia; DON (dissolved organic nitrogen) = TN - DIN (dissolved inorganic nitrogen: $NO_x$ and $NH_3$).

| | Co | Sp | Te | CoSp | CoTe | SpTe | CoSpTe |
|---|---|---|---|---|---|---|---|
| Surface area in $cm^2$ | 276 (10.7) | 515 (17.4) | 428 (18.0) | 396 (11.9) | 346 (5.3) | 457 (12.6) | 408 (11.9) |
| DOC | 54.5 (4.02) | 74.8 (17.18) | 31.0 (4.47) | 69.7 (21.31) | 47.8 (18.59) | 57.1 (9.95) | 52.6 (19.33) |
| TN | 3.2 (0.57) | 4.6 (2.78) | 3.4 (0.65) | 5.7 (1.13) | 4.0 (1.12) | 5.9 (1.50) | 5.7 (1.25) |
| $NO_x$ | 0.04 (0.009) | 0.03 (0.014) | 0.02 (0.007) | 0.05 (0.022) | 0.03 (0.018) | 0.03 (0.018) | 0.03 (0.019) |
| $NH_3$+$NH_4^+$ | 0.37 (0.315) | 0.71 (1.314) | 0.24 (0.419) | 0.10 (0.038) | 0.27 (0.256) | 0.14 (0.176) | 0.42 (0.605) |
| DON | 2.8 (0.63) | 3.9 (1.62) | 3.1 (0.43) | 5.5 (1.13) | 3.7 (1.11) | 5.7 (1.51) | 5.2 (1.32) |

**Figure 1.** Single- and mixed-species treatment effects (black) on (a) leaf litter loss and (c) C:N ratios (n=9 per treatment). The expected value of a polyculture (gray) is average of the observed responses of constituent species in monoculture. Letters over bars denote groups of similar means. · and * represent marginal and significant evidence that observed polyculture responses differ from expected ($0.05 < p < 0.1$ and $p < 0.05$ respectively). Plots of means represent overall effects of species richness on (b) leaf litter loss and (d) C:N ratios. P-values < 0.05 note significant regressions of a response with litter richness. Raw means with SE bars are presented. n=27 for 1 and 2 species richness levels; the three species level had n=9. Species abbreviations are: Co, Cocobolo, *Dalbergia retusa*; Sp, Spiny Cedar, *Pachira quinata*; and Te, Teak, *Tectona grandis*.

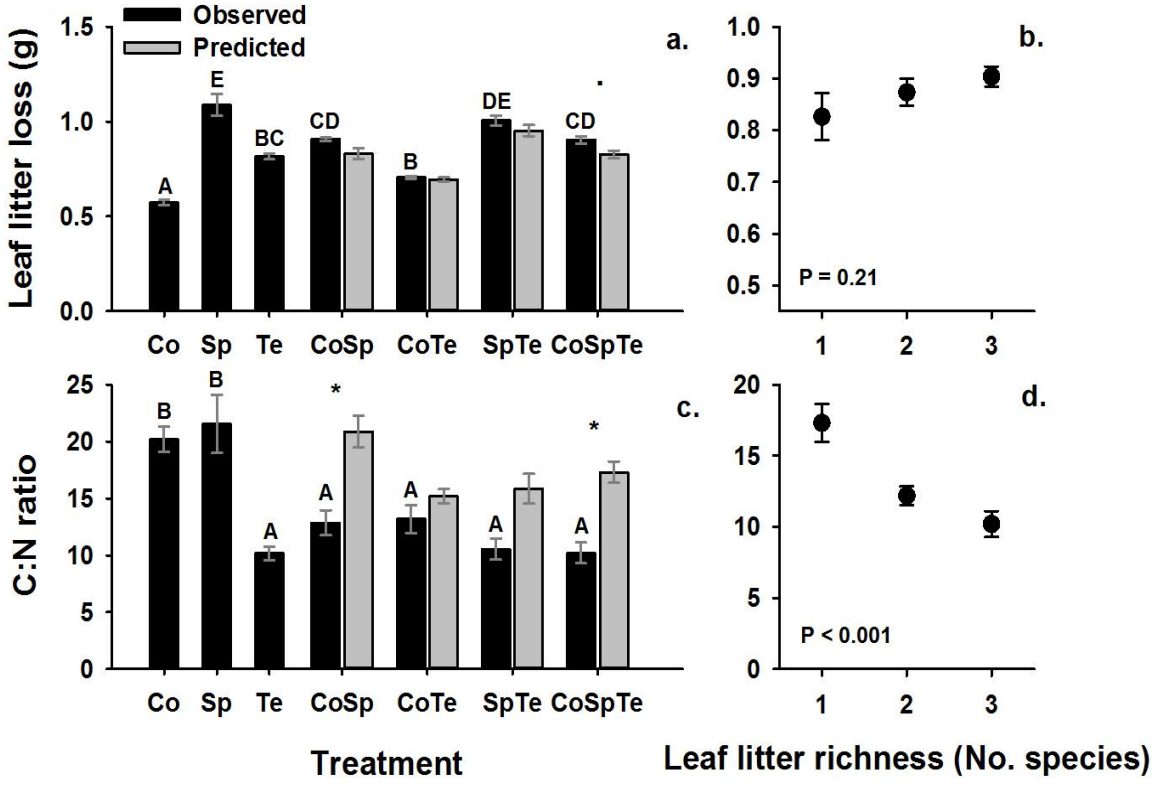


**Figure 2.** Relationship between total leaf surface area ($cm^2$) in a cup and leaf litter loss (g). p <<< 0.0001; $R^2$ = 0.75; d.f.=61; y = 0.002x-0.008.

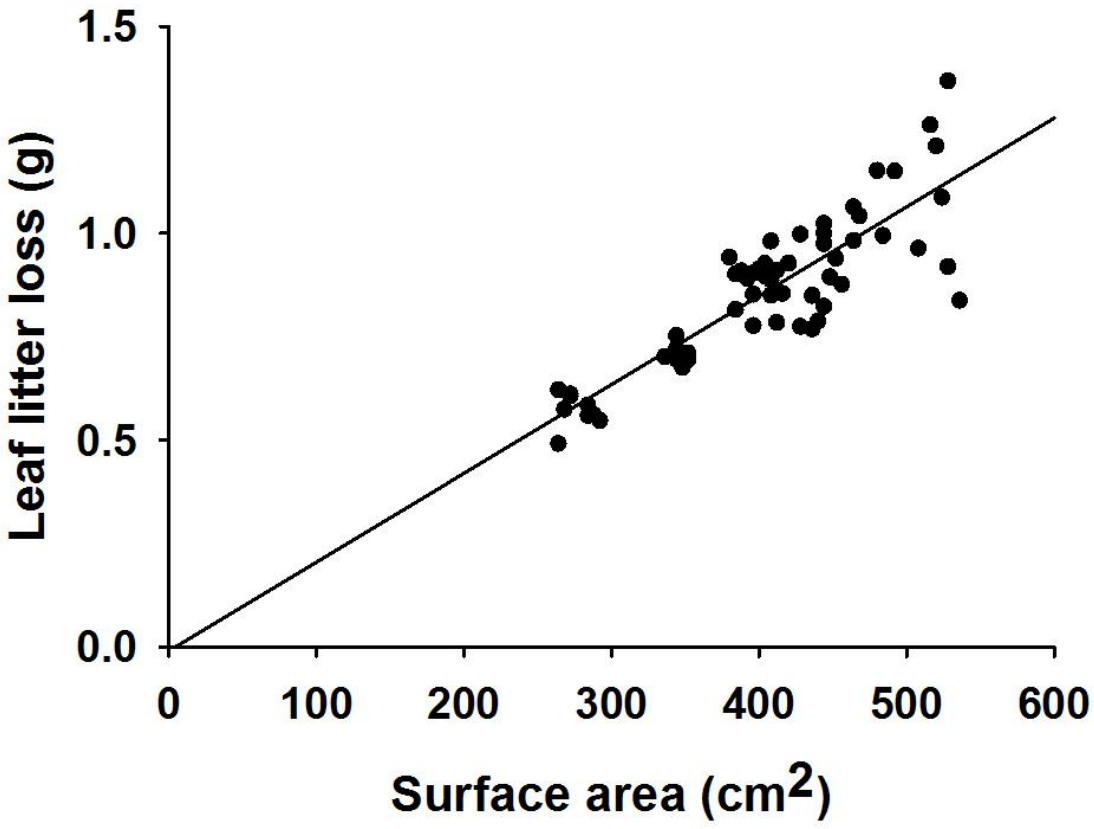


**Figure 3**. Single- and mixed-species treatment effects (in black) on (a) total abundance and (c) *Limatus* spp., (e) *Trichoprosopon digitatum*, and (g) *Culex* sp. 1 abundances. Plots of means represent overall effects of species richness on (a) total abundance and (c) *Limatus* spp., (e) *Trichoprosopon digitatum*, and (g) *Culex* sp. 1 abundances. See caption from Figure 1 for further information.

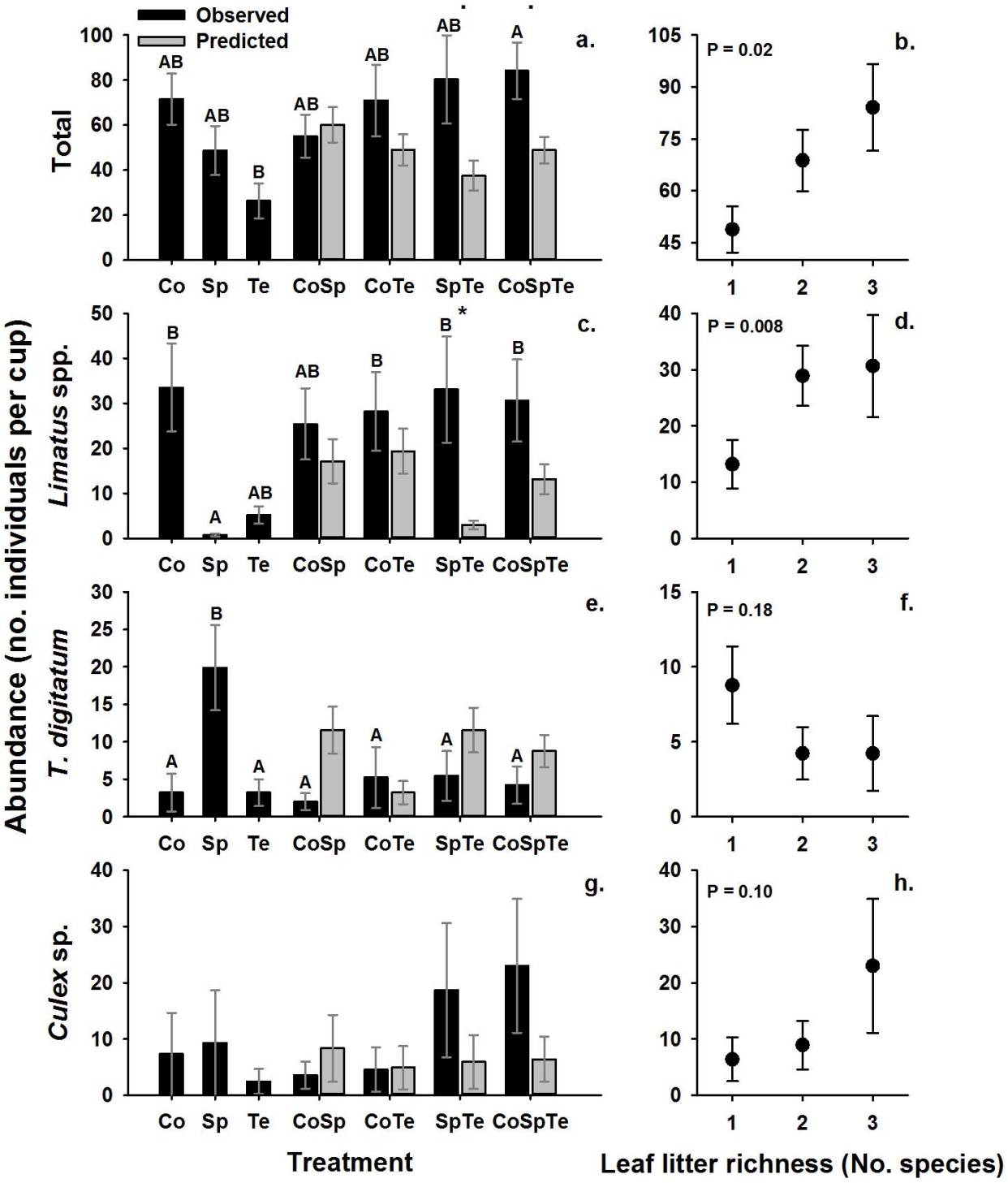

Observed
Predicted
a.
b.
c.
d.
e.
f.
g.
h.
Total
Limatus spp.
T. digitatum
Culex sp.
P = 0.02
P = 0.008
P = 0.18
P = 0.10
Co Sp Te CoSp CoTe SpTe CoSpTe
1 2 3
Abundance (no. individuals per cup)
Treatment
Leaf litter richness (No. species)

**Figure 4.** Correlation between total abundance of macroinvertebrates dissolved C:N. p = 0.27; $R^2$ = 0.02; d.f.=61.

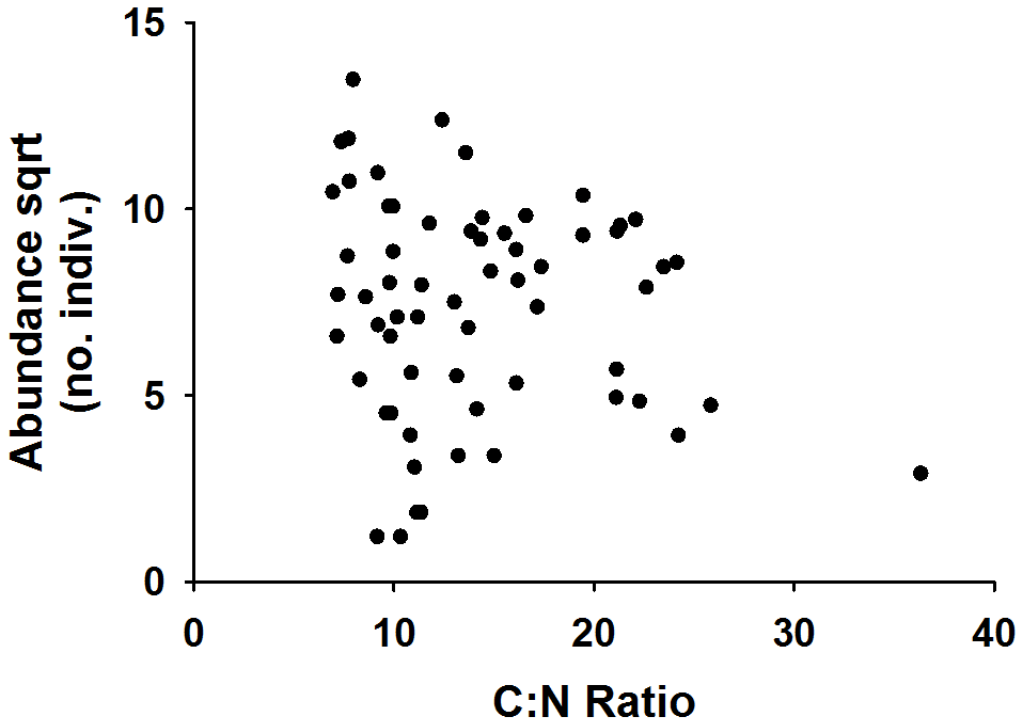


**Figure 5.** Correlation between total abundance of macroinvertebrates and dissolved organic carbon. $p = 0.07$; $R^2 = 0.05$; d.f.=61.

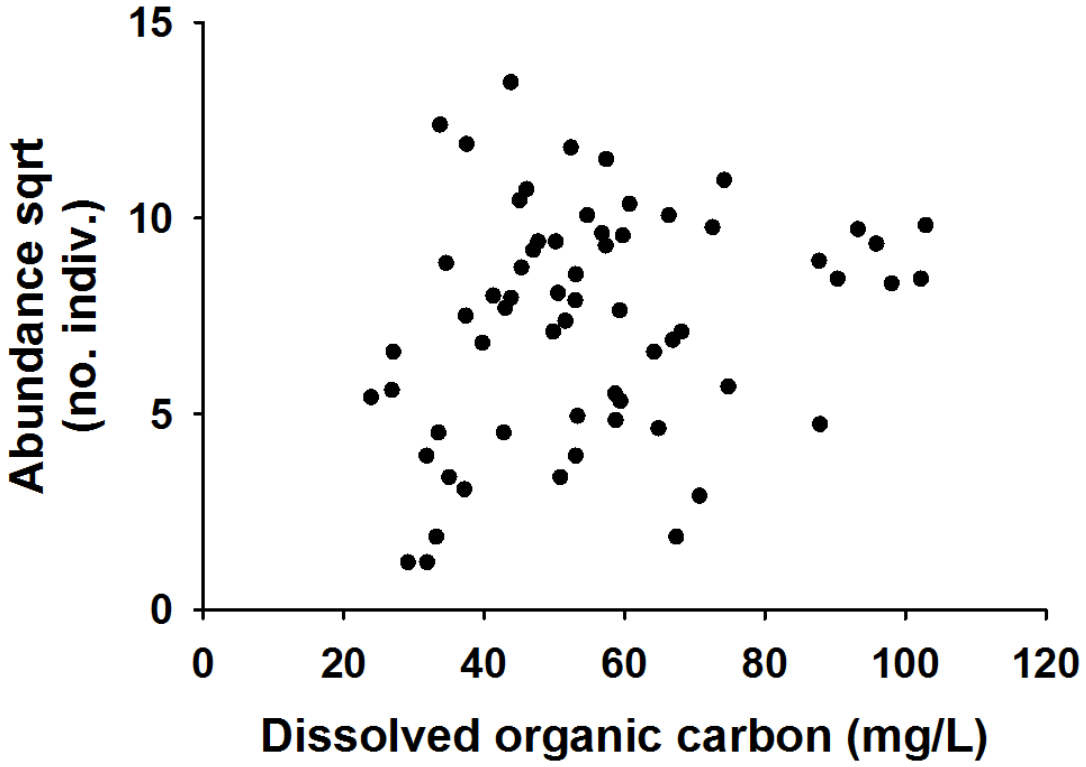


**Figure 6.** Correlation between total abundance of macroinvertebrates and dissolved organic nitrogen. p = 0.001; $R^2$ = 0.16; d.f.=61; y = 0.73x+4.27.

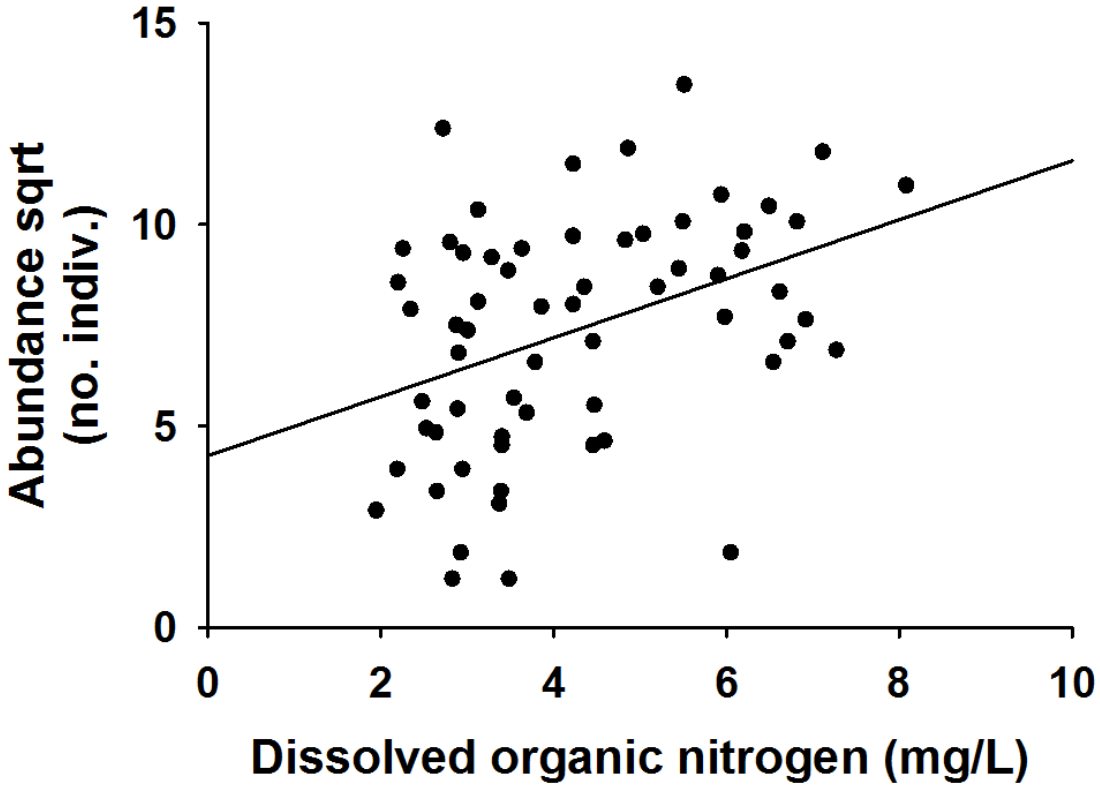
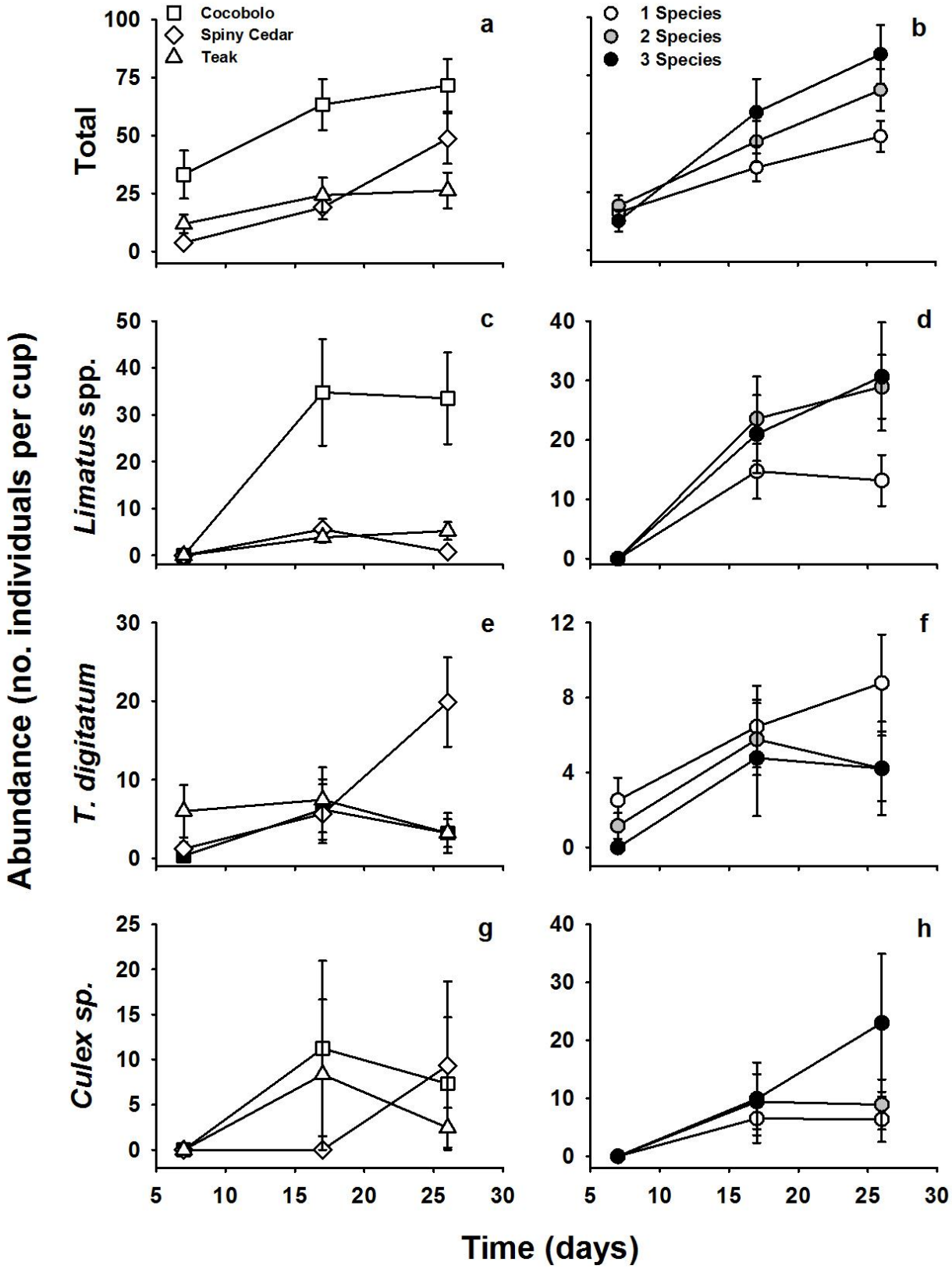


**Figure 7. (Supplemental to results)** Effects of leaf litter species and richness over the three sampling times during the study on (a, b) total abundance and taxa specific abundances: (c, d) *Limatus* spp., (e, f) *Trichoprosopon digitatum*, and (g, h) *Culex* sp.1. Plots show means with SE bars. Individual leaf litter species treatments have n=9; n=27 for the one- and two-species litter richness treatments and n=9 for the three species polyculture treatment.


Cocobolo
Spiny Cedar
Teak
1 Species
2 Species
3 Species
a
b
c
d
e
f
g
h
Total
Limatus spp.
T. digitatum
Culex sp.
Abundance (no. individuals per cup)
Time (days)

## Vita

Rachel A. Komosinski was born on June 27, 1987 in Mission Viejo, California. Her family moved to San Jose, California when she was two, where she lived until she migrated to Ohio at age nine to be close to her parents' families. She graduated from Findlay High School, her mother's alma mater, in 2005. Following high school she attended Michigan State University where she majored in Zoology with a concentration in Genetics. She received a Bachelor's degree in Science in 2009 when she graduated with high honors from the Lyman Briggs College and Honors College of MSU. She spent the next year researching predator-prey interactions in a zooplankton community in Lake Michigan and preparing for graduate school. She traveled to Richmond, VA in August 2010 and matriculated at Virginia Commonwealth University to pursue a Master's degree in Biology under the guidance and tutelage of Dr. James Vonesh. She travelled to Panama to complete her research that next summer. Following her Master's degree Ms. Komosinski will be teaching as an adjunct instructor at VCU while she becomes certified to teach high school biology.